



Original Article

Some Histochemical and Morphometric Observations on the Liver of the Dromedary Camel (*Camelus dromedarius*)

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ABSTRACT

The objectives of this study were to investigate some histochemical components and to conduct morphometrical study on the liver of the camel. Ten livers of adult dromedary camels were used. The distribution of glycogen throughout the hepatic tissue has been studied with the use of either Best's carmine, or Periodic Acid Schiff reagent (PAS). The glycogen content of the liver varied from animal to animal, and among lobes and lobules within the same liver. The left lobe and quadrate lobe contained more glycogen than the right and the caudate lobes. In some lobes, the lobules adjacent to subcapsular region of the liver had a larger amount of glycogen compared to the lobules located far from the capsule. Moreover, the cells located directly under the capsule showed intensely stained masses of glycogen. The hepatocytes contained numerous lipid droplets as demonstrated by the modified method of Lillie Ashbrun's Isopropanol Oil Red. The number of lipid droplets varied from moderate to numerous in the hepatocytes of all livers examined. The distribution of the glycogen and lipid showed a distinct lobular pattern; where the hepatocytes located at the peripheral zones of the lobules contained by far more of these two substances than the centrolobular cells. The mean absolute volume of the fresh liver was about 6692 cm³. Morphometric data of the components of the liver as percentages from the absolute volume were as follows; the hepatocytes (79.60%), the blood vessels and hepatic sinusoids (12.38%), the connective tissue (7.70%), and the bile duct and ductules (0.30%). The morphometric analysis indicated that the left hepatic lobe had the largest amount of connective tissue. Also the left lobe and quadrate lobe presented a high percentage of blood supply.

Keywords Liver, dromedary, morphometry, lipid, glycogen.

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INTRODUCTION

Glycogen and lipid could be demonstrated in the cytoplasm depending on the functional state of the liver (Nickel *et al.*, 1973). The content of

these materials in the liver might therefore vary greatly with the diet (Bloom and Fawcett, 1986). In normal mice, histochemical estimation

showed a peculiar lobular pattern of hepatic glycogen. After feeding, periportal cells showed intensely stained masses of glycogen (Hammad *et al.*, 1982). In monkey, glycogen occurred throughout the cytoplasm as small closely packed granules and no carbohydrates other than glycogen were found (Bearcroft, 1960). In healthy liver of camel and buffalo, Bahgat *et al.*, (1964) and Bahgat *et al.*, (1965) stated that, glycogen granules were evenly distributed throughout the lobule. However, Shahien *et al.*, (1977) claimed that, in some lobules, glycogen was concentrated more in the hepatocytes at the peripheral and central zones while in other lobules, glycogen is evenly distributed. Lalla and Drommer (1997) stated that, in hepatocytes containing lipid droplets, the glycogen is concentrated mainly around these droplets.

In all mammals, lipid droplets were few in normal hepatocytes, but may increase in disease, after consumption of alcohol, or toxic substance (Bloom and Fawcett, 1986). In normal rat liver, the lipid content was low and mainly localized in peripherally in the lobules (Van Noorden *et al.*, 1994). Soveri (1993) reported that, unlike the situation in cows, the reindeer calves had no fat accumulation in the hepatocytes. In pig liver, there were large cytoplasmic vacuoles in the hepatic cells, although their significance was unknown (Bojan, 1971). In the camel, Abdalla *et al.*, (1971) indicated that, large fat cells were present sporadically in the liver. However, Lalla and Drommer (1997) described a mild to moderate lipid content in normal hepatocytes. According to Shahien *et al.*, (1977), the hepatocytes contained small to medium-sized lipid droplets concentrated in the peripheral part of the cells along the sinusoids. Khatim *et al.*, (1985), on the other hand, stated that, the hepatocytes of camel were characterized by the presence of numerous cytoplasmic inclusions (vesicles, vacuoles) that might occupy most of the cell, and appeared larger than the nuclei, although their significance was unknown.

Although there are some quantitative anatomical data on the liver of certain species of animals: Sheep (Gooneratne *et al.*, 1980), Rainbow trout (*Salmo gairdneri*) (Hampton *et al.*, 1989), rat (Zhao *et al.*, 1993), Black Bengal goats and Chotanagpuri sheep (Massarat *et al.*, 1996) and man (Ludwig *et al.*, 1998), comprehensive morphometric data on the liver of the dromedary camel is virtually lacking.

The conflicting reports about the distribution of glycogen and lipid, and the absence of any reference, in the literature available to the authors, of the relative volumes of the components of the dromedary liver, the present study was undertaken as prerequisite to understanding structure- function relationship of the liver of the camel.

MATERIALS AND METHODS

A total of ten livers were collected from slaughter-houses. Specimens were obtained from both sexes at different ages. The livers used for this study were all apparently normal, and each liver was taken as soon as possible following slaughtering of the animals.

Tissue Preparation

Histochemistry

Thin slices of tissue (about 1 cm long and 5 mm thick) were taken from the right, left, quadrate and caudate lobes of the liver and from the portal fissure. For detection of glycogen, tissues were fixed either in Gender's Fluid at 0-4°C, or in 10% formalin. After fixation, the tissues were processed by routine histological techniques (Culling, 1974; Drury and Willington, 1980). The specimens were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin. Tissue blocks were then cut at a thickness between 5 and 7 µm and mounted onto glass slides pre-coated with albumin. The sections were further cleared in xylene and rehydrated in descending grades of ethanol, washed in water and stained with Best's carmine or PAS. Control sections for glycogen were treated with 0.1% diastase or saliva for 30 min, at 37°C, or at room temperature (Drury and Willington, 1980).

For demonstration of lipid droplets, tissue blocks were fixed either in 10% formal calcium or 10% formal saline for 24 hours, then frozen in liquid nitrogen (-197°C) before being cut in the cryostat at a thickness between 10 and 15 µm. The slides were rinsed in 10% formalin to fix the sections on the slides. Then they were stained by Oil Red O-triethyl phosphate method (Drury and Willington, 1980). Control sections were treated with acetone at -20°C for 20 minutes to extract lipid (Pearse, 1965).

Morphometry

For morphometric study, five normal livers were used. Volumes of fresh livers were determined by water displacement method (Aherne and Dunnill, 1982). Five thin slices were taken randomly from the right, left, quadrate and caudate lobes and from the portal fissure of each liver. Samples were processed for routine histological technique and stained with Haematoxylin and Eosin according to Culling (1974). A total of twenty five sections from five livers were used for morphometric analysis. A grid with 100 points, fitted in $\times 12.2$ eye piece, was used to determine the volume densities of the main components of the liver (Aherne and Dunnill, 1982). These components are: hepatocytes, blood vessels, connective tissue, interlobular bile ductules and ducts. Each section was entirely analyzed, field by field, giving a range of 15-33 fields per section. The sufficiency of the number of points necessary to count for each component in order to keep the standard error below 5% was confirmed by the plot of Weibel (1963). The absolute volumes of the components of the liver were calculated from the volume densities (V_v) of the component, and the total volume (V) of the fresh liver. The statistical analysis of the data obtained by the point-counting was restricted to the calculation of the mean and standard deviation (Weibel, 1963).

RESULTS

Glycogen

The distribution of glycogen in the cytoplasm of the hepatocytes appeared either as scattered fine granules or as small closely packed granules (Fig. 1).

The glycogen content of hepatocytes varied from cell to cell. In some cells, the glycogen clumps had a perisinusoidal arrangement (Fig. 1). Following the use of Best's carmine method, the distribution of glycogen was uniform throughout the lobule (Fig. 1). However, the hepatic glycogen showed a distinct lobular pattern when PAS method was used. Within the lobule, the peripheral hepatic cells showed intensely stained masses of glycogen (Fig. 2). The area of the peripheral zone varied among lobules, depending on the amount of glycogen. In some lobules, the mass of glycogen appeared as a thin strand while in other lobules it was thick (Fig. 2).

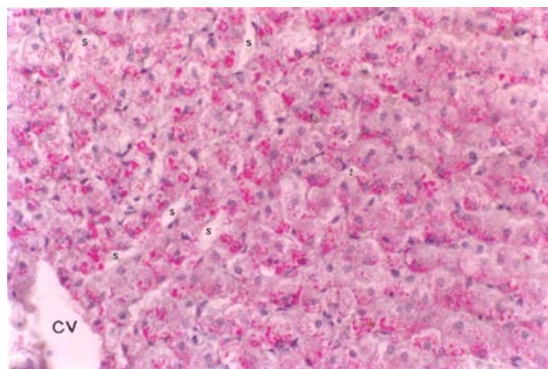


Fig. 1: Photomicrograph showing hepatic cords and central vein (CV). Note that the glycogen granules are mostly present in the peripheral cytoplasm of the hepatocytes along the sinusoids (S). Best's carmine. X 400.

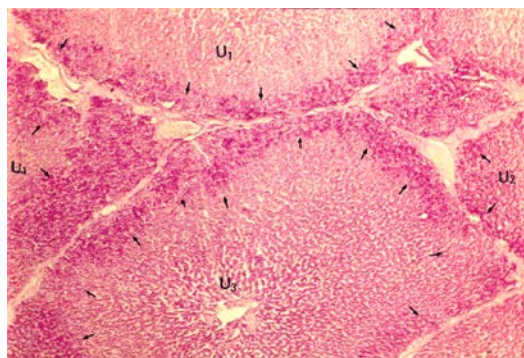


Fig. 2: Photomicrograph showing that the area of the peripheral zone (arrows) varies between the different lobules ($U_{1,2,3,4}$). PAS X 100.

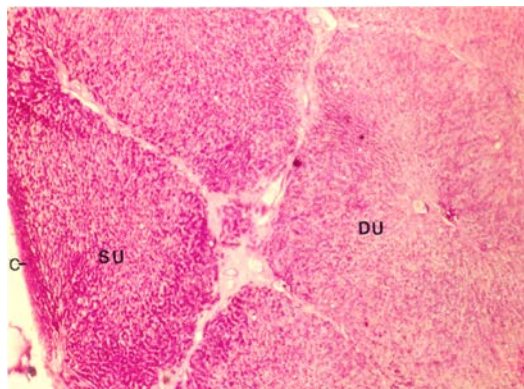


Fig. 3: Photomicrograph demonstrating that the glycogen granules in superficial lobules (SU). C: capsule stain more intensely than those in lobules located deeply (DU). PAS. X40.

The lobules located at subcapsular region of the liver had a larger amount of glycogen compared to the lobules present away from the capsule (Fig. 3 and 4). It was clearly distinguished that the glycogen content of the liver varied from animal to animal, and among

lobes within the same liver. The present study showed that, the left and quadrate lobes contained more glycogen than the right and caudate lobes. In these two lobes, the hepatocytes of the middle and peripheral zones of some lobules contained larger amount of glycogen than the central zones (Fig. 5).

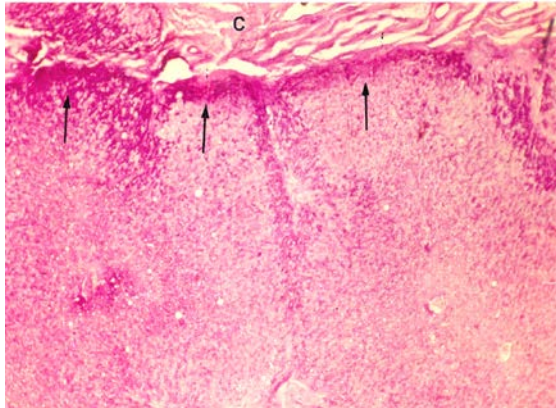


Fig. 4: Photomicrograph illustrating masses of glycogen granules (arrow) in one part of peripheral zones of lobules, which situated under capsule (C), stain more intensely than the deeper parts of the same lobules. PAS. X100

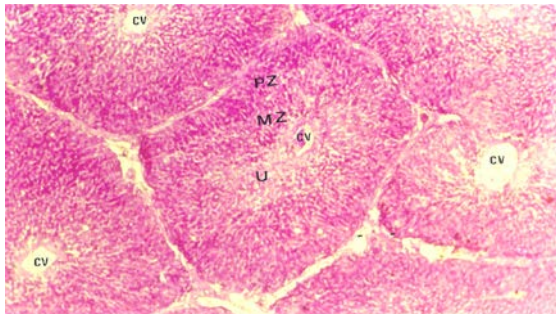


Fig. 5: Photomicrograph from the left lobe illustrating the hepatocytes of both middle and peripheral zones of lobules showing more intensely-stained masses of glycogen than in the cells of the central zone. CV: Central vein. PAS. X40.

Lipids

The current investigation showed that, the hepatocytes of the liver of the camel were characterized by the presence of numerous cytoplasmic vacuoles in ordinary histological slides (Fig. 6). The used of the modified staining method of Lillie Ashbrun's Isopropanol Oil Red for lipid demonstrated a moderate to a large number of lipid droplets in the cytoplasm of the hepatocytes. The lipid droplets were distributed all over the lobule but they had tendency to be concentrated in the cells located at the peripheral zone than in the cells of the central zone (Fig. 7). The lipid droplets varied in size, and located in the cytoplasm of hepatocytes adjacent to the sinusoids (Fig. 8).

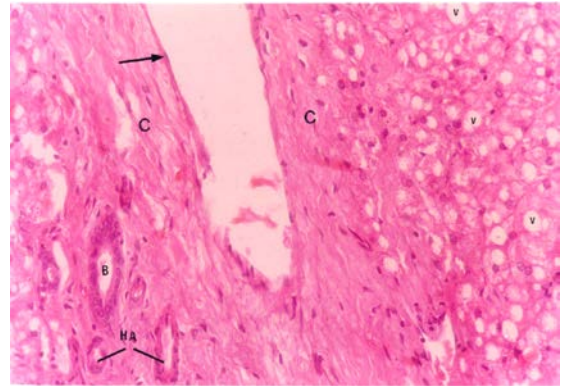


Fig. 6: Photomicrograph showing the capsule (C) covered by serous membrane with flattened nuclei (arrow) and containing a small bile duct (B) and branches of hepatic artery (HA). The hepatocytes show spherical vacuoles (V) of varying size. H and E. X 250.

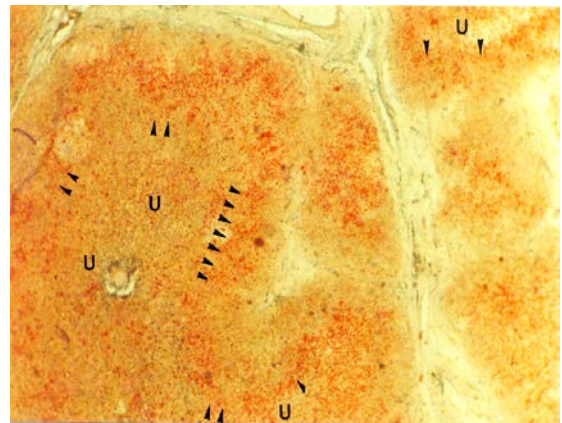


Fig. 7: Photomicrograph demonstrating Lipid droplets distributed all over the lobule (U) but are more concentrated at the cells of the peripheral zone (arrowheads). Oil Red O. X40.

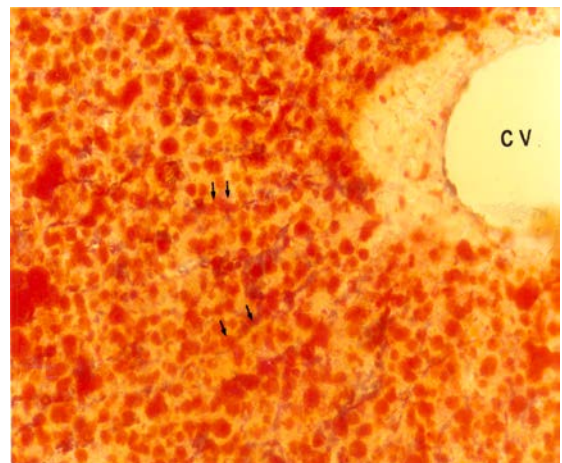


Fig. 8: A higher magnification from the photomicrograph in Fig. 7 showing lipid droplets of varying sizes, within the hepatocytes, and are situated in the peripheral cytoplasm of the cells adjacent to the sinusoids (arrows). CV: central vein. Oil Red O. X250.

Table 1: Showing the volume of fresh livers and the volume densities of their main components in 5 camels expressed as mean percentages and standard deviations. Mean absolute volume of these components are indicated in cm^3

Animal No.	Volume of fresh livers	Hepatocytes		Blood vessels and sinusoids		Bile duct and ductules		Connective tissue	
		Cm^3	%	Cm^3	%	Cm^3	%	Cm^3	%
1	9480	7679.70	81.01±6.31	1055.10	11.13±3.77	19.91	0.21±0.11	725.22	7.65±2.89
2	7840	6087.76	77.65±4.17	993.33	12.67±2.8	14.11	0.18±0.099	744.8	9.50±1.97
3	4620	3810.11	82.47±2.69	535.00	11.58±1.99	13.40	0.29±0.11	261.49	5.66±0.84
4	9320	7334.84	78.71±1.31	1230.24	13.20±1.23	27.96	0.33±0.13	726.96	7.75±1.67
5	2200	1719.74	78.17±2.60	293.04	13.32±2.19	10.12	0.46±0.21	177.1	8.05±1.5
Mean±SD	6692	5326.23±2522.80	79.60±3.42	821.34±391.30	12.38±2.4	17.1±7.02	0.294±0.13	527.11±282.68	7.72±1.77

Table 2: Showing the volume fractions of the main components of the right lobe of the liver of 5 camels expressed as mean percentages and standard deviations (SD).

Camel No.	Hepatocytes (%)	Blood vessels and sinusoids (%)	Bile duct and ductules (%)	Connective tissue (%)
1	85.05	9.81	0.08	5.06
2	80.43	10.67	0.23	8.67
3	84.18	10.39	0.39	5.04
4	77.35	13.9	0.30	8.45
5	78.29	12.71	0.48	8.52
Mean	81.060	11.49	0.296	7.148
SD	3.45	1.73	0.15	1.92

Table 3: Showing the volume densities of the main components of the right, left, quadrate and caudate lobes, and the porta hepatis in 5 camels expressed as mean percentages and standard deviations

Lobes of the liver	Hepatocytes	Blood vessels and sinusoids	Bile duct and ductules	Connective tissue
Right lobe	81.1%±3.45	11.5%±1.73	0.3%±0.15	7.1%±1.92
Left lobe	70.6%±4.09	14.0%±3.165	0.3%±0.05	9.7%±2.01
Quadrate lobe	78.3%±4.59	13.6%±2.82	0.3%±0.096	7.8%±2.97
Caudate lobe	80.3%±2.18	12.4%±1.11	0.3%±0.27	7.0%±1.52
Porta hepatis	82.4%±2.64	10.4%±1.78	0.3%±0.19	6.9%±1.38

Morphometric Study

Tables (1-3) were showing the results of the morphometric analysis. The mean volume of the fresh liver of the camel (*Camelus dromedarius*) was about 6692 cm^3 .

The morphometric data showed that, the mean percentages of the volume densities of the main components of the liver were: the hepatocytes (79.60±3.42), the blood vessels and hepatic sinusoids (12.38±2.4), the connective tissue which included the capsule, trabeculae, and portal canals (7.70%±1.77), the bile duct and ductules (0.30%±0.13) (Tables 1 and 2).

From the morphometric analysis, the left hepatic lobe contained the largest amount of interlobular and intralobular connective tissue (9.7%), while the left lobe and quadrate lobe presented a high percentage of blood vessels (14.0%) (Table 3).

DISCUSSION

The present investigation showed that, the glycogen content of the dromedary liver as a whole was variable, varying from cell to cell, and from lobule to lobule. These observations confirmed the earlier findings of Bahgat *et al.*, (1965) and Lalla and Drommer (1997) in the camel. The distribution of the glycogen indicated a distinct lobular pattern where the hepatocytes located at the peripheral zones of all lobules and both peripheral and intermediate zones in some lobules showed more intensely-stained masses of glycogen compared to the hepatocytes localized at the centrolobular zones. This confirmed earlier observations in the liver of man (Leeson and Leeson, 1970; Kudryavtseva *et al.*, 1996). However, Shahien, *et al.*, (1977) claimed that, in the dromedary

liver, in some lobules, glycogen is concentrated more in the hepatocytes located at both peripheral and central zones than in other lobules, in which glycogen is evenly distributed. In the rat liver, the hepatocytes of the central zone showed a higher concentration of glycogen than in the other zones (Van Noordeen *et al.*, 1994). The conflicting reports about the distribution of glycogen may be explained by stating that glycogen is a very labile substance. Another probable explanation is offered by Jungerman and Kietzmann (1996) who claimed that, although the liver tissue was uniform on the level of histology, it was heterogeneous on the level of morphometry and histochemistry. This heterogeneity was due to the blood supply. Cells located in the upstream or periportal zone differ from those in the downstream or central zone in their equipment with key enzymes, translocator, receptors and subcellular structures and therefore, had different metabolic zonation.

In this study, the glycogen content of the liver varied from animal to animal, and among lobes within the same liver. The left and quadrate lobe contained more glycogen than the right and the caudate lobes. This result confirmed the explanation made by Scheiff (1931), who attributed the variation in the glycogen content of the different parts of the liver to differences in the blood supply of the various lobes. Interestingly, the present morphometric findings of the dromedary liver showed that, the left and quadrate lobes had a volume of blood vessels higher than that in other lobes. In contrast, Corrin and Aterman (1968) stated that, glycogen content in the liver of rats, fasted and re-fed at various intervals, is normally uniform in distribution; they attributed the variations in the content of glycogen between lobes, reported by other authors, to the techniques of sampling and determination. On the other hand, Shahien *et al.*, (1977) claimed that, the histochemically demonstrable glycogen showed a marked dependence on food intake. Yang and Makita (1998) reported that, with decreasing weight of livers, the glycogen content decreases in fasting group of Japanese monkeys.

In all mammals, lipid droplets are few in normal hepatocytes, but may increase in disease, after consumption of alcohol, or toxic substance (Bloom and Fawcett, 1986). Abdalla *et al.*, (1971) claimed that large fat cells are present sporadically in the liver. Lalla and Drommer

(1997) described a mild to moderate lipid infiltration in normal hepatocytes. In this investigation, the results tend to confirm the findings of Lalla and Drommer (1997) except that in this study the fat content of hepatocytes was moderate to large. According to Shahien *et al.*, (1977), the hepatocytes of the liver of the camel contained small to medium-sized lipid droplets concentrated in the peripheral part of the cells along the sinusoids. Khatim *et al.*, (1985) on the other hand, reported that, the hepatocytes of camel were characterized by the presence of numerous cytoplasmic inclusions (vesicles, vacuoles) that may occupy most of the cell, and appear larger than the nuclei. It is probable that these 'vacuoles' or 'vesicles' reported by Khatim *et al.*, (1985) are lipid droplets extracted during preparation of the slides. In the present investigation, the lipid droplets were found in all hepatocytes but more concentrated in the hepatocytes at the periphery of the lobules. Similar distribution of lipid droplets has been reported in the liver of the rat (Van Noordeen *et al.*, 1994).

Mirgani (1981) stated that, the value of total lipids in camel liver is 27.3 mg/g unit tissue, and increases to 36.7 mg/g unit tissue after fasting for 11 days. Uro (1987) has observed an increase in total lipids in camel livers in well fed animals. Steinberg (1963) has already explained that by suggesting the presence of an active fatty acid cycle. Thus the high concentration of lipid content of the liver of the camel in this study may be a result of fattening or fasting before slaughter, or an indication of a special fat metabolism in the camel. As in ruminants, volatile fatty acids are produced in the forestomach of the camel (Holler *et al.*, 1989), but like animals with simple stomach, the camel maintains a high level of blood glucose (Uro, 1986). In addition to that, the activities of citrate cleavage enzymes, malic enzyme, and fatty acid synthetase are much higher in the liver of camel than in the liver of ruminant (Mirgani *et al.*, 1987; Uro, 1987). The above mentioned reports, and that of Lalla and Dromer (1997), and the present study, may suggest an important role played by the liver of camel in lipogenesis.

Noteworthy, The liver acini is roughly an oval area of parenchyma encompassing portions of two classic lobules. It is supplied by the terminal branches of the portal vein and hepatic artery, and is drained by a terminal branch of

bile duct. A central vein is at each end of the acinus, and a portal area on one side (Dellmann and Brown, 1981; Bloom and Fawcett, 1986). Three zones of varying metabolic activity between the axes and the central vein become evident (Passmore and Robson, 1971; Arey, 1974; Banks, 1981). The presence of structural and functional units (acini) has been demonstrated in man, dog and rabbits (Rappaport, Borowy, Lougheed and Lotto, 1954; Rappaport, 1958). However, the structure of acinus is never found in the pig liver (Bhunchet and Wake, 1998). In the normal, and in the fibrotic rat liver, the existence of liver acini and classic lobule is doubtful (Bhunchet and Wake, 1998).

It is pertinent to mention here that the distribution of glycogen and lipids in the liver of the camel in this study strongly supports the idea of the liver acinus (Passmore and Robson, 1971; Arey, 1974 and Banks, 1981).

The findings of this study showed that the absolute volume of the liver of the camel was about 6692 cm³. The investigation also showed that, in percentage volume and in descending order, the components of the liver rated as follows: hepatocytes (79.6%), blood vessels (12.4%), connective tissue (7.7%) and bile ducts (0.3%). The investigation also showed that the percentage volume of the components varied between the liver lobes. For example, the left lobe had a larger amount (9.7%) of interlobular and intralobular connective tissue than the other lobes. It is clear from the present investigation that the liver of the camel had a high content of fibrous tissue. It is known that the liver of the pig is less friable than that of any other domestic mammal because of its high content of interlobular connective tissue (Sisson and Grossman, 1975). In the absence of any reference, in the literature, of the relative volumes of the components of the liver in the pig, it is not possible to state with any certainty which of the two species, camel or pig, has the higher content of connective tissue. The percentage volume of the intrahepatic bile ducts in this study, and in other reports, is interesting. Firstly, it appears that the percentage volume is the same whether the animal possesses a gallbladder (man) or not (camel, rat). It is also interesting, as revealed in this study, that the percentage volume of the intrahepatic bile ducts was similar in the various lobes of the liver,

despite the observation that the other components may differ in percentage volume between lobes. For example, the left lobe had the highest content of interlobular and intralobular connective tissue. It is also of interest to mention that in the liver of the camel, the left and quadrate lobes had a volume of blood vessels (14.0%) higher than in other lobes.

CONCLUSION

The present study confirmed the fact that although the liver of the camel was uniform on the level of the histology, it was heterogenous on the level of morphometry and histochemistry. The normal hepatocytes in the camel contained a large amount of lipids, a feature which suggests a probable role of the liver in lipogenesis.

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